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Assessment of Toxicity, Genotoxicity and Toxicological Mechanism for Selected Bioactive Compounds Present in *Bacopa monnieri* Linn. by using ProTox II Webserver

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Abstract: ***Bacopa monnieri Linn. is commonly known as Bramhi in Bengali and found in marshy area, which is an important medicinal plant as per traditional knowledge. This plant contains several phytochemicals, which are used for anti - Alzheimer's agent. The present study predicted physico - chemical properties and different types of toxicity parameters viz. acute toxicity (rat oral LD_{50}), hepatotoxicity, immunotoxicity, genotoxicity endpoints viz. cytotoxicity, carcinogenicity, mutagenicity, as well as toxicological mechanism especially stress response pathway of selected phytochemicals of B. monnieri and synthetic medicine (Donepezil) by using ProTox - II webserver. The phytocompounds Memantine and Bacopaside N2 were found class III, categorized as toxic if swallowed. All studied compounds did not show hepatotoxic while 4 phytocompounds Scopoletin, Bacopasaponin G, Bacopaside B and Bacopaside N2 and synthetic medicine viz. Donepezil were obtained immunotoxic active. Regarding genotoxicity, two compounds viz. Bacopaside N2 and Donepezil were found cytotoxic while Scopoletin and Donepezil were obtained carcinogenic, but all compounds were mutagenic inactive. All studied compounds were found inactive for nrf2/ARE, HSE, p53 and ATAD5 while 4 phytocompounds viz. Catechin, Galangin, Bacopasaponin G and Bacopaside N2 were observed MMP active. It is concluded that Bacopasaponin G was found to be better efficacious than Donepezil like drug. In future study, it is suggested to conduct in vivo bioassay to validate these predictive results.***

Keywords: Anti - Alzheimer's agent, *Bacopa monnieri*, Predictive toxicity, *In silico*, Toxicological mechanism

1. Introduction

The Indian medicinal plant, *Bacopa monnieri* Linn. is commonly known as Bramhi in Bengali and found in marshy area. [1] As per traditional knowledge, this plant is used for Ayurvedic medicine from past 3000 years especially for the prevention of neurological disorders. [2, 3] In past research, several studies have been documented that the bioactive compounds present in this plant prevent different neurological disorders. [4 - 13]

Moreover, the extract of *B. monnieri* was observed to cause side effects in the gastrointestinal tract, i. e., nausea, increased stool frequency and abdominal cramps. [8] An earlier case report revealed that severe liver toxicity observed in a woman after taking several Ayurvedic herbs, including *B. monnieri*, for the period of nine months; while she stopped taking the herbs, her liver function returned to normal. [14] Muchhara et al. [21] examined the safety of Bacognize®, a standardized botanical extract obtained from the whole herb *Bacopa monnieri* (L.) Wettst., where they did not observe genotoxicity of this botanical.

But some authors described that plant extract may contain allelochemicals, which is toxic to animals. In this context, prior to the usage of extract, toxicity screening is utmost concern and *in silico* study helps faster screening, no animal harming and inexpensive method. [19]

The present study was attempted an *in silico* approach to screen physico - chemical properties, toxicity, genotoxicity endpoints, toxicological mechanisms of established phytochemicals of *B. monnieri* compared to synthetic medicine (Donepezil).

2. Materials and Methods

All established phytochemicals of *Bacopa monnieri* Linn. and synthetic medicine viz. Donepezil was taken from available literature. [20 - 23]

Banerjee et al. [19] developed the ProTox - II platform in which physico - chemical properties and different types of toxicity parameters viz. acute toxicity (rat oral LD_{50}), [20] hepatotoxicity, immunotoxicity, genotoxicity endpoints viz. cytotoxicity, carcinogenicity, mutagenicity, as well as toxicological pathway such as stress response pathway is classified five target - pathway based models such as nuclear factor (erythroid - derived 2) - like 2/antioxidant responsive element (ARE), heat shock factor response element (HSE), mitochondrial membrane potential (MMP), phosphoprotein tumor suppressor (p53), and ATPase family AAA domain - containing protein 5 (ATAD5) were evaluated.

3. Results

Table 1 describes the values of predictive physico - chemical parameters of natural and synthetic compounds. Three phytochemicals such as Bacopasaponin G, Bacopaside N2 and Bacopaside C were observed >500gm/mol molecular weight.

Table 1: Prediction of physico - chemical properties of natural and synthetic compounds

Sl. No.	Compounds name	MolWt (gm/mol)	HBD (nos.)	HBA (nos.)	A (nos.)	B (nos.)	R (nos.)
1.	Catechin	290.7	5	20	35	37	1
2.	Galangin	270.24	3	14	30	32	1
3.	Scopoletin	192.17	1	11	22	23	1
4.	Memantine	179.3	1	22	34	36	0
5.	Bacopasaponin G	736.93	6	76	116	123	6
6.	Bacopaside A	370.42	4	35	50	50	10
7.	Bacopaside B	492.47	6	39	63	65	10
8.	Bacopaside N2	796.98	8	82	124	131	7
9.	Bacopaside C	536.53	6	44	70	73	11
10.	Donepezil	379.49	0	33	57	60	6

MolWt = Molecular weight; HBD = Hydrogen bond donor; HBA = Hydrogen bond acceptor; A = Atoms; B = Bonds and R = Rings

Table 2 describes the values of predictive oral acute toxicity, class and accuracy of natural and synthetic compounds. The phytocompounds Memantine and Bacopaside N2 were found class III, categorized as toxic if swallowed.

Table 2: Prediction of oral acute toxicity, class and accuracy of natural and synthetic compounds

Sl. No.	Compounds name	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1.	Catechin	10000	VI	100
2.	Galangin	3919	V	70.97
3.	Scopoletin	3800	V	100
4.	Memantine	157	III	100
5.	Bacopasaponin G	2000	IV	69.26
6.	Bacopaside A	4000	V	68.07
7.	Bacopaside B	5000	V	70.97
8.	Bacopaside N2	55	III	69.26
9.	Bacopaside C	3000	V	69.26
10.	Donepezil	505	IV	68.07

Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class VI: non - toxic ($LD_{50} > 5000$)

Table 3 describes the values of predictive systemic toxicity of natural and synthetic compounds. All studied compounds did not show hepatotoxic while 4 phytocompounds Scopoletin, Bacopasaponin G, Bacopaside B and Bacopaside N2 and synthetic medicine viz. Donepezil were obtained immunotoxic active.

Table 3: Prediction of systemic toxicity of natural and synthetic compounds

Sl. No.	Compounds name	H	P	I	P
1.	Catechin	Ic	0.72	Ic	0.96
2.	Galangin	Ic	0.68	Ic	0.97
3.	Scopoletin	Ic	0.69	Ac	0.54
4.	Memantine	Ic	0.90	Ic	0.99
5.	Bacopasaponin G	Ic	0.95	Ac	0.99
6.	Bacopaside A	Ic	0.71	Ic	0.93
7.	Bacopaside B	Ic	0.81	Ac	0.99
8.	Bacopaside N2	Ic	0.93	Ac	0.99
9.	Bacopaside C	Ic	0.89	Ic	0.69
10.	Donepezil	Ic	0.98	Ac	0.95

H = Hepatotoxicity; I = Immunotoxicity; Ic = Inactive; Ac = Active and P = Probability

Table 4 describes the values of predictive genotoxicity endpoints of natural and synthetic compounds. Two compounds viz. Bacopaside N2 and Donepezil were found cytotoxic while Scopoletin and Donepezil were obtained carcinogenic, but all compounds were mutagenic inactive.

Table 4: Prediction of genotoxicity endpoints of natural and synthetic compounds

S. No.	Compounds name	C	P	M	P	Cr	P
1.	Catechin	Ic	0.84	Ic	0.55	Ic	0.51
2.	Galangin	Ic	0.98	Ic	0.52	Ic	0.72
3.	Scopoletin	Ic	0.91	Ic	0.56	Ac	0.53
4.	Memantine	Ic	0.70	Ic	0.69	Ic	0.61
5.	Bacopasaponin G	Ic	0.57	Ic	0.91	Ic	0.74
6.	Bacopaside A	Ic	0.81	Ic	0.64	Ic	0.77
7.	Bacopaside B	Ic	0.79	Ic	0.83	Ic	0.81
8.	Bacopaside N2	Ac	0.56	Ic	0.91	Ic	0.77
9.	Bacopaside C	Ic	0.76	Ic	0.80	Ic	0.81
10.	Donepezil	Ac	0.63	Ic	0.53	Ac	0.50

C = Cytotoxicity; M = Mutagenicity; Cr = Carcinogenicity; Ic = Inactive; Ac = Active and P = Probability

Table 5 describes the values of predictive stress response pathways of natural and synthetic compounds. All studied

compounds were found inactive for nrf2/ARE, HSE, p53 and ATAD5 while 4 phytocompounds viz. Catechin, Galangin, Bacopasaponin G and Bacopaside N2 were observed MMP active.

Table 5: Prediction of stress response pathways of natural and synthetic compounds

Sl. No.	Compounds name	nrf2/ ARE	P	HSE	P	MMP	P	p53	P	ATAD5	P
1.	Catechin	Ic	0.94	Ic	0.94	Ac	0.55	Ic	0.95	Ic	0.98
2.	Galangin	Ic	0.99	Ic	0.99	Ac	1.0	Ic	0.92	Ic	0.92
3.	Scopoletin	Ic	0.96	Ic	0.96	Ic	0.53	Ic	0.87	Ic	0.75
4.	Memantine	Ic	0.99	Ic	0.99	Ic	0.94	Ic	0.98	Ic	0.99
5.	Bacopasaponin G	Ic	0.95	Ic	0.95	Ac	0.70	Ic	0.67	Ic	0.56
6.	Bacopaside A	Ic	0.91	Ic	0.91	Ic	0.89	Ic	0.91	Ic	0.95
7.	Bacopaside B	Ic	0.96	Ic	0.96	Ic	0.92	Ic	0.90	Ic	0.97
8.	Bacopaside N2	Ic	0.88	Ic	0.88	Ac	0.62	Ic	0.72	Ic	0.64
9.	Bacopaside C	Ic	0.93	Ic	0.93	Ic	0.78	Ic	0.85	Ic	0.96
10.	Donepezil	Ic	0.93	Ic	0.93	Ic	0.78	Ic	0.85	Ic	0.96

nrf2/ARE = Nuclear factor (erythroid - derived 2) - like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour supressor); ATAD5 = ATPase family AAA domain - containing protein 5; Ic = Inactive; Ac = Active and P = Probability

4. Discussion

In the present study, the phytocompounds Memantine and Bacopaside N2 were found class III, categorized as toxic if swallowed in rat oral LD_{50} value. In past study by Subhan et al., [24] the hydroethanolic extract of *B. monnieri* observed LD_{50} value of 232 mg/Kg in mice, which may be due to these two compounds and comparatively higher than the present data. In rat model, the extract of 5000 mg/Kg of the *B. monnieri* did not show toxic effects. [25]

In this *in silico* study, all studied compounds did not show hepatotoxic, but an earlier case report indicated that severe liver toxicity observed in a woman after taking several Ayurvedic herbs, including *B. monnieri*, for the period of nine months; while she stopped taking the herbs, her liver function returned to normal. [14]

Regarding genotoxicity endpoints of natural and synthetic compounds, two compounds viz. Bacopaside N2 and Donepezil were found cytotoxic while Scopoletin and Donepezil were obtained carcinogenic, but all compounds were mutagenic inactive. A similar finding by Muchhara et al. [21] revealed that the whole herb *Bacopa monnieri* (L.) Wettst. Did now show mutagenicity. examined the safety of Bacognize®, a standardized botanical extract obtained from where they did not observe genotoxicity of this botanical.

In earlier study, Roy et al. [26] predicted that two saponins viz. Bacopasaponin G compared to Donepezil on CASP - 3 and TPK I receptors showed favourable binding energy value where these phytocompound obtained inhibitory effect on the studied receptors and this small molecule may lead compound to prevent Alzheimer's disease. Herein, Bacopasaponin G observed non - toxic and non - genotoxic in the present toxicity prediction.

5. Conclusion

In conclusion, among these phytocompounds and synthetic medicine, Bacopasaponin G was found to be better efficacious than Donepezil like drug. In future study, it is suggested to conduct *in vivo* bioassay to validate these predictive results.

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Conflict of interest
No conflict of interest during study and manuscript preparation.

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